NON-INHERITANCE OF IMPRESSED VARIATIONS IN STREPTOCOCCUS LACTICUS.*

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MICROORGANISMS should furnish unusually favorable material for a study of the immediate effect of environment upon cell characteristics, both physiological and morphological, and of the inheritance of the characters thus impressed. Bacteria in particular should prove to be valuable assets in the study of heredity on account of their rapidity of multiplication, the complete absence of the complications introduced by sexual reproduction, the relatively simple structure of the cell, and the intimate relationship necessarily existing between the protoplasm and the chemical and physical factors of the environment. These advantages are in part offset by the minuteness of the cells and the difficulty in isolation and recognition of individuals.

The present study was suggested by the considerable variation noted in the lactic acid production of the various starters used in the college dairy. The original purpose was to develop, if possible, a type of lactic acid organism that would produce an unusually large amount of lactic acid in milk, and to test the organism thus obtained in the commercial manufacture of butter. It was deemed advisable to use statistical methods as far as possible in recording the data obtained, both as an index of progress and for convenience in summarizing results. The need of such methods was particularly emphasized by the appearance of the papers of Goodman[1] on acid production in the diphtheria group and of Winslow and Walker[2] on the paratyphoid bacillus.

REVIEW OF PREVIOUS WORK.

The literature on the subject of variation in bacteria is voluminous, and numerous deductions as to the inheritance of characters may be found recorded. These latter in many cases will not stand careful analysis. In few instances have the data been

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† Mr. Truax made most of the culture transfers, titrated the greater portion of the samples, and assisted in the compilation of the data.

[1] *Jour. Infect. Dis.*, 1908, 5, p. 240. [2] *Ibid.*, 1909, 6, p. 90.

sufficient in quantity or of the right character to justify wholly the conclusions reached. The discussion here given of the work of others and of their conclusions is by no means exhaustive, as it has been thought best to include only the more noteworthy of the investigations and of the theories of heredity in microorganisms.

The work of investigators may for convenience be divided into studies on inheritance of morphological variations and on morphological mutants and into those that have to do with physiological variations. In few instances have careful statistical studies been made.

Beijerinck[1] secured an asporogenous race of Schizosaccharomyces octosporus by plating a pure culture and selecting those colonies in which no spores were to be observed. He found that the ability to produce spores did not return even after long cultivation under favorable conditions. Winogradsky[2] noted a similar loss of spore-producing power in Clostridium pastorianum when this organism was grown for some time on potato. Lepeschkin[3] succeeded in isolating races of Schizosaccharomyces pombe and S. melacei with a characteristic myceloid type of colony. These he considered to be true mutations in the De Vriesian sense, and not simply developmental stages in the growth of the organism. Later (1904) he reported a somewhat similar study on inheritance of branching in the cells of B. Berestnewi. Through selection he secured cultures in which the proportion of branched cells was greatly increased. He argues that a very direct relationship exists between the environment and the appearance of branching, and that cultivation in certain media tends to increase the amount of specific determinative material in the cell. This is then passed on its unusual quantities to the daughter cells. Hansen[4] by his work upon yeasts has demonstrated that the progeny of a single cell may develop into both top and bottom yeasts, and that selection may be used to fix this character. He also secured mutants, from the progeny of a single cell, that lost the ability to produce spores, and this power of spore production was not regained through cultivation under favorable conditions during many years. He believes the mutations observed to be brought about by definite changes in the environment. Barber[5] has shown that certain cells of the B. coli of unusual shapes, when isolated and cultivated, transmit the peculiarities in the few cases in which growth could be induced. These unusual shapes were not common, and many times failed to develop, but in several instances races were obtained from pure cultures that differed materially from the parent type. Garbowski[6] has contributed a most valuable statistical study of the immediate effect of environment upon the morphology of bacteria, particularly upon the cell dimensions. He gives no evidence, however, that these impressed variations may be transmitted. Jennings[7] has shown that in the Paramoecium there exists many pure races that remain true to type. He finds that mass selection may be used to modify mass characteristics, but this is accomplished by elimination of some of the races and not by the development of new characters. Changes in environment do not seem to modify permanently the morphology of a pure race, nor does continuous selection of individuals alter the

[1] *Centralbl. f. Bakt.*, Abt. 2, 1897, 3, pp. 449, 518.

[2] *Ibid.*, 1902, 9, p. 43.

[3] *Ibid.*, 1903, 10, p. 145; 1904, 12, p. 641; 13, p. 13.

[4] *Ibid.*, 1905, 15, p. 353; 1907, 18, p. 577.

[5] *Kan. Univ. Sci. Bull.*, 1907, 4, p. 3.

[6] *Centralbl. f. Bakt.*, Abt. 2, 1907, 19, pp. 641, 737.

[7] *Jour. Exp. Zool.*, 1908, 5, p. 577; *Amer. Nat.*, 1909, 18, p. 321; *ibid.*, 1910, 44, p. 136.

type. This work on a protozoan is a verification for these forms of the pure line theories developed by Johannsen in his work with plants. Clark[1] attempted to modify the morphology of various members of the diphtheria group; particularly did he try to convert the non-virulent type into the virulent. All his efforts failed to modify these characteristics in any degree. Each of the strains with which he worked maintained its own characteristic morphology with remarkable constancy.

There are numerous records of modifications of bacteria as to physiological and pathogenic characters. A review of the latter phase of the subject would scarcely be profitable. We have good reason to believe that virulence may be exalted or diminished in many organisms but by no means in all. Clark for example attempted to increase the virulence of the pseudo-diphtheria bacillus by repeated inoculations and isolations under what he believed to be the most favorable conditions, but he failed to modify the virulence to an appreciable degree. Color modifications have also been a favorite source material for studies of variation and inheritance in bacteria, with the most conflicting results. It seems entirely probable that in some cases true mutations and heritable modifications have been observed. Indol and gas production in members of the intestinal group and gas production in yeasts have been studied by various investigators (Peckham,[2] Horrocks,[3] Hartmann,[4] Massini,[5] Twort,[6] Burri[7]). It is difficult to estimate to just what extent the modifications observed by these writers are due to the selective action of environment on chance modifications or mutations, and to what extent to its influence on the mass of organisms. Careful statistical studies would seem to be required for this determination, for at present authors are by no means in agreement.

Goodman[8] selected a single colony of the diphtheria bacillus of a type producing an acidity of 2 per cent normal in dextrose broth, and made transfers to 15 tubes of sugar broth. The reaction of each tube was determined by titration at the end of three days, and a new series of 15 tubes inoculated from the tube showing the highest acidity and a similar series from those showing lowest acidity. Each of these, designated respectively the high and low series, was carried through 36 transfers. At the end of this time the maximum difference in reaction between the two series had risen to about 5 per cent of normal acid, and the difference in the means to $3\frac{9}{10}$ per cent. Goodman regards this as evidence that gradual modifications in the physiological characteristics may be cumulative and ultimately result in very considerable differences in the extremes. Winslow and Walker carried out a somewhat similar series of experiments in which they used two strains of the paratyphoid bacillus. The investigation "was planned so as to exclude the . . . . factor, the direct effect of environment and to deal with the inheritance of spontaneous variations of the fluctuating type." Each culture was plated and a hundred colonies isolated on agar, and from each agar tube one of 1 per cent dextrose broth was inoculated. Those tubes were chosen from which the culture showing the highest acid production in each series had been inoculated and from these new plates were poured, isolations made on agar and broth tubes inoculated from these as before, and their acidity determined. A third series was carried through in the same manner. The frequency polygons of each series were then plotted and compared. The selection seemed to be without observable effect, the polygons showed little or no evidence of change. These results in a certain measure may be opposed to Goodman's findings, altho the conditions of experimentation differed

[1] *Jour. Infect. Dis.*, 1910, 7, p. 335.
[2] *Jour. Exper. Med.*, 1897, 2, p. 549.
[3] *Jour. Roy. Army Med. Corps*, 1903, 1, p. 362.
[4] *Wchnschr. f. Brauerei*, 1903, 20, p. 113.
[5] *Centralbl. f. Bakt.*, Abt. 1, 1906, 38, p. 98.
[6] *Ibid.*, Ref. 1907, 42.
[7] *Ibid.*, Abt. 1, 1910, 54, p. 210.
[8] *Loc. cit.*

in two cases. In these cases there do not seem to have been intrinsic heritable variations, and it would seem that such variations must exist in the diphtheria group or must be impressed upon the organisms by environment, otherwise there would be nothing upon which selection could act.

MATERIALS AND METHODS.

The original purpose of this investigation, as has been stated, was twofold, first to develop, if possible, a high acid race of the Strept. lacticus for experimental work, and second, to study statistically the effect of selection upon acid production of the Strept. lacticus. Milk, buttermilk, cream, and commercial starters of various types were plated in deep litmus lactose agar in various dilutions to obtain the source cultures for the work. These were incubated at blood heat and the acid colonies isolated as they appeared. Colonies of Strept. lacticus only were isolated. Altogether 20 cultures from as many different sources were selected for the work. The lactose broth used was prepared at two different times, about 20 liters each time. Every effort was made to keep all the media as uniform in composition as possible. The customary procedure for the preparation of sugar-free broth from beef was followed. The broth was made neutral to phenolphthalein, autoclaved in liter flasks, and preserved for use as needed. Five per cent of lactose was added to these as required, and placed in test tubes and sterilized in the Arnold for 15 minutes on each of three successive days. The tubes after inoculation were kept in the thermostat at blood heat for three days and then titrated while hot with phenophthalein as an indicator against twentieth normal sodium hydrate. Blanks were kept with all cultures, and were titrated and their acidity (varying from 0.8 to 1 per cent normal acid) subtracted from the recorded acidities of the inoculated tubes. Plating was in all cases upon 1 per cent lactose agar.

TABULATION OF RESULTS.

Cultures of the Strept. lacticus from 20 sources were secured, as has been stated, by the isolation of colonies developing upon the litmus lactose agar. The diagnosis of the organism was made in every instance by its ability to coagulate milk, and by stained mounts. A transfer was then made of each culture to a tube of lactose broth, incubated for three days, and 5 c.c. titrated as above. Table 1 gives the results of this titration.

TABLE 1.

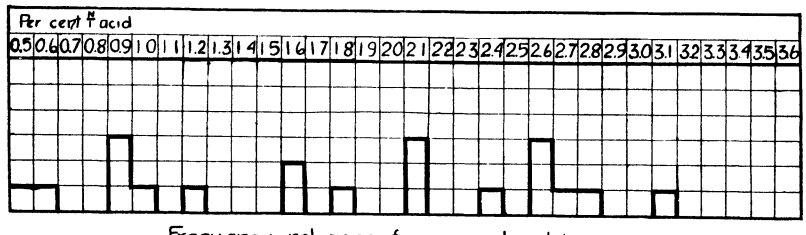
PERCENTAGE N/1 PRODUCED BY THE 20 ORIGINAL CULTURES OF STREPT. LACTICUS.

Culture	Percentage N/1 Acid	Culture	Percentage N/1 Acid
A	3.05	K	2.05
B	.94	L	1.64
C	2.71	M	2.61
D	1.79	N	No growth
E	2.64	O	.95
F	.48	P	1.64
G	2.81	Q	1.23
H	2.56	R	.90
I	2.05	S	2.35
J	.89	T	.62

The very considerable variation in the amount of acid produced is more clearly shown by the frequency polygon in Chart 1. In this series, as in all succeeding, the titrations were read to the second decimal place, but in preparing charts and tables the reading is usually to the nearest tenth.

The lowest of the series is F with 0.48 per cent N/1 acid and the highest A with 3.05 per cent. From each of these tubes plates

CHART 1.



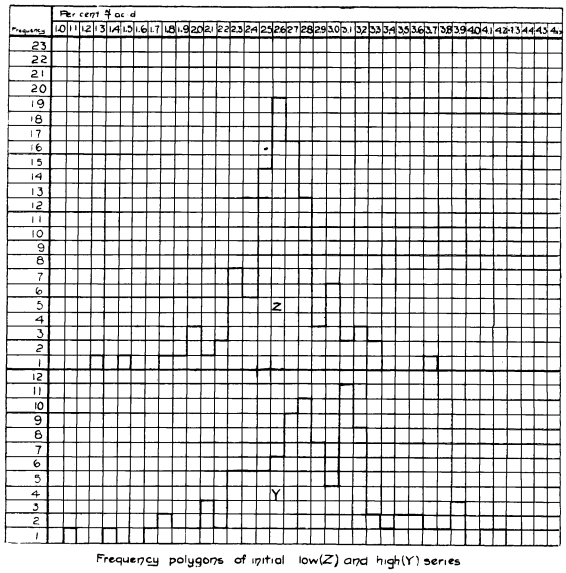
Frequency polygons for original isolations.

were poured in litmus lactose agar and incubated at 37° C. On the second day 100 tubes of lactose broth were inoculated from individual colonies, these were incubated, and titrated after 72 hours. This was done that a frequency polygon showing the distribution in acid production of individuals from each might be plotted for purposes of comparison and to serve as a standard that would record any subsequent deviation. For convenience in reference the high series is called Y and the low series Z. The first two columns of Table 3 give the distribution, mean and standard deviation, or index of variability for each series. Chart 2 gives the distribution of the population in each case in the form of a frequency polygon.

An examination of these figures and of the chart reveals an unexpected uniformity. The means differ by an appreciable degree 0.27 and the mean of the transfers from the high acid culture Y is higher than that from the transfers from the low acid culture Z. The difference is much less, however, than one might expect from the differences in the source cultures. It is noteworthy also that the lowest of either series is 0.6 per cent N/1 acid higher than the low original. Possibly too sudden changes in the osmotic

tension of the environing media may have inhibited growth to some extent in the low type, and equilibrium was not established sufficiently soon to allow normal acid production in three days in the original tubes. The highest and lowest acid cultures of each of these series were used for the inoculation in each instance of 10 lactose broth tubes. These were designated Low Y, High Y, Low Z, and High Z (abbreviated to LY, HY, LZ, and HZ). These were titrated after incubation and a new series of 10 tubes each

CHART 2.



Frequency polygons of initial low(Z) and high(Y) series

inoculated from the low acid tubes of the LY and the LZ, and from the high acid tubes of HY and HZ. These in turn furnished the basis for a new set. Transfers were made into 23 such sets of 40 in an endeavor to "breed" high and low acid races from Y and Z by continual selection of those cultures showing the desired characteristics. The method followed in its essentials was that of Goodman. Table 2 gives the results of these titrations.

To facilitate comparisons, the frequency polygons for acid production in these cultures have been plotted in Charts 3*a*, 3*b*, and 3*c*.

A study of these diagrams emphasizes two points in particular. First, changes in environment influence markedly the amount of acid produced. When cultures LY_5, HY_5, LZ_7, and HZ_7 were in the thermostat, the temperature fell to about 18° C. and an exami-

TABLE 2.

PERCENTAGE N/1 ACID IN LY, HY, LZ, AND HZ SERIES.

	LY_1	HY_1	LZ_1	HZ_1	LY_2	HY_2	LZ_2	HZ_2	LY_3	HY_3	LZ_3	HZ_3
	2.49	2.49	2.14	2.69	1.71	2.24	1.90	2.20	2.17	2.19	2.73	2.56
	2.17	2.21	2.69	3.01	2.80	1.80	2.72	1.21	2.05	1.79	2.77	2.24
	2.29	2.09	2.98	2.78	2.81	1.96	2.78	2.08	2.84	2.36	2.81	2.19
	2.40	2.65	2.85	2.70	2.07	1.95	3.06	1.37	2.65	2.45	2.78	2.12
	2.51	2.57	2.70	2.86	1.93	2.15	2.56	2.55	2.33	2.35	1.74	2.13
	2.25	2.29	2.65	2.56	2.01	2.33	3.00	2.54	2.57	2.06	2.62	2.16
	2.43	2.08	2.61	2.56	2.50	1.91	3.20	2.97	2.46	2.12	2.48	2.41
	2.79	2.68	3.20	3.55	1.38	2.30	3.20	2.98	2.44	2.20	2.65	2.70
	2.70	2.61	2.77	3.11	2.67	2.59	3.00	2.95	2.67	2.90	2.82	2.61
	2.69	2.42	2.73	1.98	2.53	1.79	2.80	2.92	....	2.21	2.78	2.55
Means.....	2.472	2.409	2.732	2.680	2.241	2.102	2.822	2.377	2.464	2.263	2.718	2.367

	LY_4	HY_4	LZ_4	HZ_4	LY_5	HY_5	LZ_5	HZ_5	LY_6	HY_6	LZ_6	HZ_6
	2.17	1.92	1.91	2.30	2.29	2.16	2.41	2.80	2.27	2.15	2.69	2.93
	2.12	2.09	2.30	2.36	2.20	2.02	2.54	2.78	2.43	2.67	2.75	2.80
	2.38	2.36	1.98	2.40	1.70	2.00	2.54	2.62	2.49	2.70	2.83	2.84
	2.10	2.55	2.34	2.27	1.98	2.14	2.32	2.60	2.51	2.66	2.85	2.99
	2.30	2.68	2.16	2.36	1.95	2.16	2.70	2.49	2.59	2.82	2.92	3.02
	2.47	2.50	2.15	2.37	1.95	2.34	2.53	2.70	2.58	2.68	3.02	2.49
	2.28	2.39	2.01	2.00	2.15	2.43	2.55	2.78	2.32	2.52	2.73	2.68
	2.09	2.25	2.28	2.18	2.15	2.21	2.61	2.49	2.50	2.63	2.99	2.98
	2.32	2.33	2.20	2.00	2.02	2.10	2.40	2.90	2.51	2.57	2.67	3.13
	2.20	2.15	2.16	2.29	1.68	2.27	....	....	2.67	2.33	2.69	2.65
Means.....	2.243	2.322	2.149	2.523	2.007	2.183	2.511	2.684	2.487	2.573	2.814	2.851

	LY_7	HY_7	LZ_7	HZ_7	LY_8	HY_8	LZ_8	HZ_8	LY_9	HY_9	LZ_9	HZ_9
	1.95	2.48	2.19	1.61	2.30	2.46	3.31	3.31	2.37	2.56	2.85	2.46
	2.48	....	1.79	1.63	2.38	2.68	3.24	3.24	2.65	2.89	2.89	2.30
	2.48	2.81	1.90	1.86	2.41	3.26	3.33	3.33	2.55	2.84	2.71	2.36
	2.39	2.44	1.81	2.09	3.01	2.88	3.14	3.14	3.07	2.68	2.52	2.88
	2.50	2.46	1.89	1.81	2.94	2.77	3.35	3.33	2.26	2.82	2.65	2.92
	2.12	2.45	1.86	1.99	3.24	2.25	3.31	3.31	2.87	2.99	3.02	2.99
	2.38	2.43	1.74	1.74	2.75	2.72	3.05	3.05	2.77	2.68	2.90	2.11
	2.26	2.58	1.97	2.09	2.63	2.55	3.09	3.09	2.88	2.92	2.90	2.55
	2.32	2.41	2.45	1.69	3.10	2.47	3.12	3.12	2.63	2.91	3.05	2.90
	2.60	2.65	2.58	2.13	2.78	2.65	3.16	3.16	3.00	3.08	2.95	2.71
Means.....	2.348	2.523	2.018	1.864	2.754	2.664	3.214	3.208	2.705	2.837	2.844	2.618

	LY_{10}	HY_{10}	LZ_{10}	HZ_{10}	LY_{11}	HY_{11}	LZ_{11}	HZ_{11}	LY_{12}	HY_{12}	LZ_{12}	HY_{12}
	3.22	3.20	2.60	2.64	2.81	2.82	2.67	2.67	2.58	2.80	2.70	2.82
	3.05	3.17	2.65	2.52	3.24	2.43	2.70	2.67	2.47	2.67	2.83	2.83
	3.08	3.41	2.70	2.29	2.72	2.79	2.66	2.84	2.76	2.78	2.55	2.87
	3.18	3.34	2.65	2.37	2.98	2.69	2.74	2.75	2.94	2.95	2.71	2.80
	3.18	3.26	2.35	2.89	3.17	2.65	2.26	2.78	2.76	2.88	2.83	2.80
	3.33	3.03	2.47	2.62	3.15	3.11	2.53	2.64	2.75	2.92	2.76	2.90
	3.10	3.02	2.39	2.23	2.96	2.98	2.78	2.59	2.93	2.89	2.79	2.74
	3.00	3.37	2.40	2.86	3.09	2.72	2.62	2.44	3.38	2.84	2.55	2.55
	2.98	3.13	2.31	3.10	3.00	2.88	2.59	2.78	2.40	2.71	2.51	2.74
	2.77	3.30	2.85	2.70	2.98	2.55	2.54	2.79	3.01	2.95	2.88	2.66
Means.....	3.089	3.223	2.537	2.622	3.010	2.762	2.609	2.695	2.798	2.839	2.711	2.771

TABLE 2.—*Continued.*

	LY_{13}	HY_{13}	LZ_{13}	HZ_{13}	LY_{14}	HY_{14}	LZ_{14}	HZ_{14}	LY_{15}	HZ_{15}	LZ_{15}	HZ_{15}
	2.22	2.48	3.05	3.05	2.64	2.55	2 50	3.15	2.59	2.57	2.50	3.00
	2.45	2.57	3.05	2.70	2.62	2.71	2.43	2.82	2.30	2.55	2.76	2.62
	2.89	2.85	2.96	2.91	2.71	2.80	2.94	2.80	2.46	2.50	2.69	3.02
	2.78	2.55	3.07	2.84	2.73	2.79	2.59	2.40	2.38	2.41	2.78	2.74
	2.99	2.56	2.94	2.80	2.77	2.60	2.88	2.25	2.56	2.17	3.07	2.87
	2.81	2.84	3.13	2.97	2.82	2.64	2.43	2.75	2.63	2.69	2.82	2.64
	2.85	2.50	2.66	2.88	2.90	2.56	2.69	2.50	2.63	2.29	2.77	2.73
	2.65	2.30	2.93	2.86	2.76	2.80	3.06	2.47	2.76	2.57	2.56	2.90
	2.84	2.72	3.16	2.94	2.68	2.58	2.88	2.49	2.52	2.73	2.33	2.75
	2.90	3.13	3.10	2.87	2.69	2.71	2.58	3.25	2.56	2.67	2.48	....
Means.....	2.738	2.650	3.005	2.882	2.732	2.674	2.648	2.688	2.539	2.515	2.676	2.807

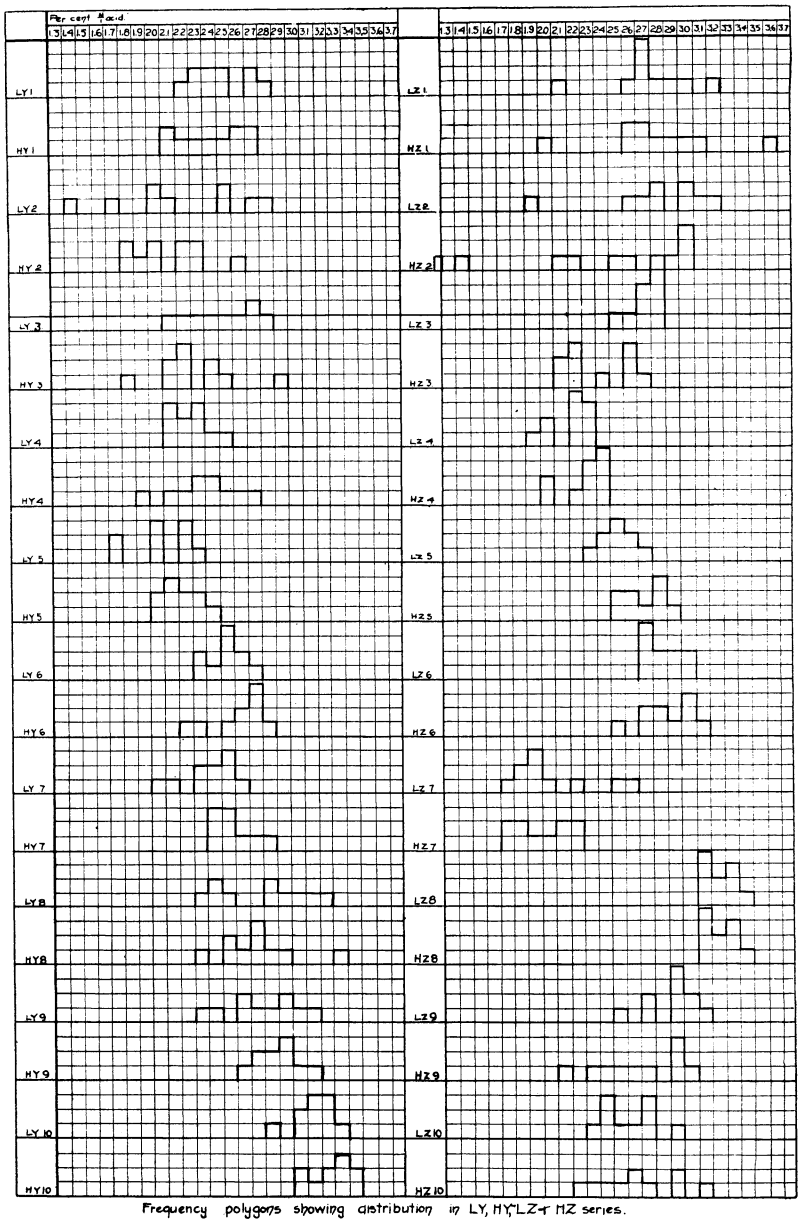
	LY_{16}	HY_{16}	LZ_{16}	HZ_{16}	LY_{17}	HY_{17}	LZ_{17}	HZ_{17}	LY_{18}	HY_{18}	LZ_{18}	HZ_{18}
	2.56	2.46	2.99	2.50	2.45	1.99	2.82	2.35	2.58	2.59	2.44	2.41
	2.46	3.26	2.92	2.51	2.88	2.71	2.49	2.34	2.54	2.53	2.70	2.60
	2.73	3.24	2.76	2.53	2.68	2.00	2.88	2.61	2.66	2.68	2.80	2.66
	2.67	3.05	2.45	2.40	2.84	2.95	2.10	2.94	2.70	2.81	2.82	3.06
	2.74	2.29	2.65	2.73	3.12	2.65	1.76	2.36	2.69	2.81	2.75	2.96
	3.04	2.44	2.86	2.77	2.77	2.78	2.67	2.19	2.32	2.63	2.64	2.96
	2.34	2.48	2.94	2.30	2.81	2.61	2.30	2.72	2.65	2.56	2.59	2.62
	2.61	2.56	3.00	2.40	2.46	2.74	2.43	2.89	2.31	2.63	2.70	2.81
	2.60	2.38	2.89	1.86	2.38	2.45	2.69	2.63	2.70	2.20	2.69	2.70
	2.70	2.41	2.80	2.83	2.47	3.00	2.61	....	2.53	2.60	2.81	2.63
Means.....	2.645	2.657	2.826	2.633	2.686	2.588	2.474	2.626	2.568	2.604	2.694	2.741

	LY_{19}	HY_{19}	LZ_{19}	HZ_{19}	LY_{20}	HY_{20}	LZ_{20}	HZ_{20}	LY_{21}	HY_{21}	LZ_{21}	HZ_{21}
	2.20	2.90	2.74	2.70	2.46	2.49	2.84	2.45	2.34	3.03	2.43	2.58
	2.78	2.93	2.76	2.54	2.91	2.56	2.94	2.29	2.99	2.27	2.50	2.43
	2.73	2.66	2.77	2.64	3.02	2.82	2.35	2.67	2.83	2.63	2.61	2.90
	2.99	2.76	2.50	2.64	2.98	2.98	2.57	2.62	2.51	2.29	2.52	2.79
	2.69	2.82	2.64	2.77	2.67	2.84	2.83	2.89	2.38	2.69	2.45	2.70
	2.24	2.91	2.89	2.71	2.90	3.07	2.99	2.73	2.51	2.93	2.78	2.69
	2.53	2.81	2.77	2.83	2.95	2.60	2.89	2.45	2.85	2.76	2.64	2.90
	2.84	2.87	2.70	2.98	2.85	2.20	2.91	2.80	2.66	2.80	2.55	2.84
	2.68	2.41	2.97	2.65	2.96	2.80	2.55	2.99	2.57	2.83	2.71	2.86
	2.78	2.65	....	2.94	2.77	2.80	....	2.71	3.02	2.68	2.52	2.83
Means.....	2.646	2.772	2.751	2.740	2.847	2.724	2.761	2.660	2.666	2.681	2.571	2.753

	LY_{22}	HY_{22}	LZ_{22}	HZ_{22}	LY_{23}	HY_{23}	LZ_{23}	HZ_{23}
	2.79	2.90	2.61	2.49	2.19	2.46	2.43	2.47
	3.09	2.87	2.80	2.77	2.72	2.82	2.83	2.68
	2.74	2.80	2.67	2.54	2.82	2.62	2.74	2.83
	3.06	2.62	2.63	2.75	2.68	2.63	3.07	2.93
	2.80	2.80	2.60	2.47	2.73	2.72	2.82	2.90
	2.78	2.39	2.60	2.74	2.75	2.70	2.92	2.95
	2.90	2.31	2.70	2.87	2.85	2.60	2.60	2.97
	2.99	2.80	2.81	2.86	2.90	2.66	2.79	2.54
	3.01	2.89	2.72	2.78	2.86	2.59	2.64	2.93
	3.07	2.41	2.88	2.69	2.99	....	....	2.73
Means.....	2.919	2.679	2.702	2.696	2.749	2.642	2.484	2.794

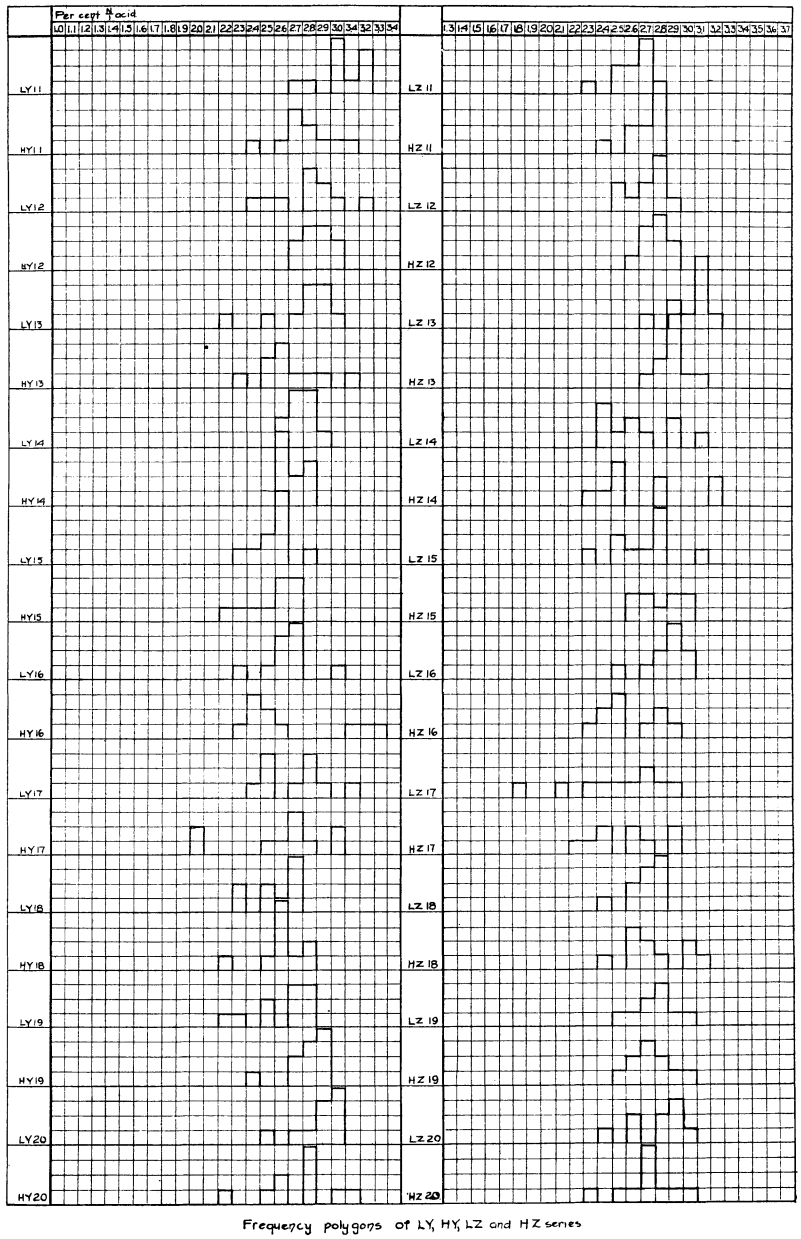
nation of the diagrams will show a considerable diminution in the amount of acid produced by these cultures. The next cultures show an increase again and a return to normal. At other times the temperature remained quite constant, yet there is to be noted a considerable variation in the amount of acid produced in the

CHART 3a.



Frequency polygons showing distribution in LY, HY, LZ & MZ series.

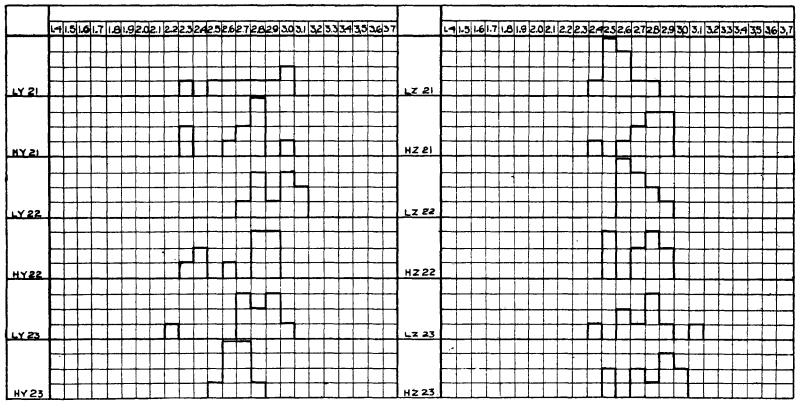
CHART 3*b*.



Frequency polygons of LY, HY, LZ and HZ series

various cultures. This is shown more clearly in Charts 4 and 5, where the maxima, minima, and means of each set of each series are plotted. None of the conditions of the experiment seem to account for the variation observed, altho it is undoubtedly due to some uncontrolled factors of the environment. Second, there is a very marked parallelism between the LY and HY series and the LZ and HZ series, and between the entire Y and Z series. Charts 4 and 5 show that there is a constantly close approximation of the means, maxima, and minima. There is certainly no such difference observable as would be expected if the series tended to diverge in

CHART 3c.

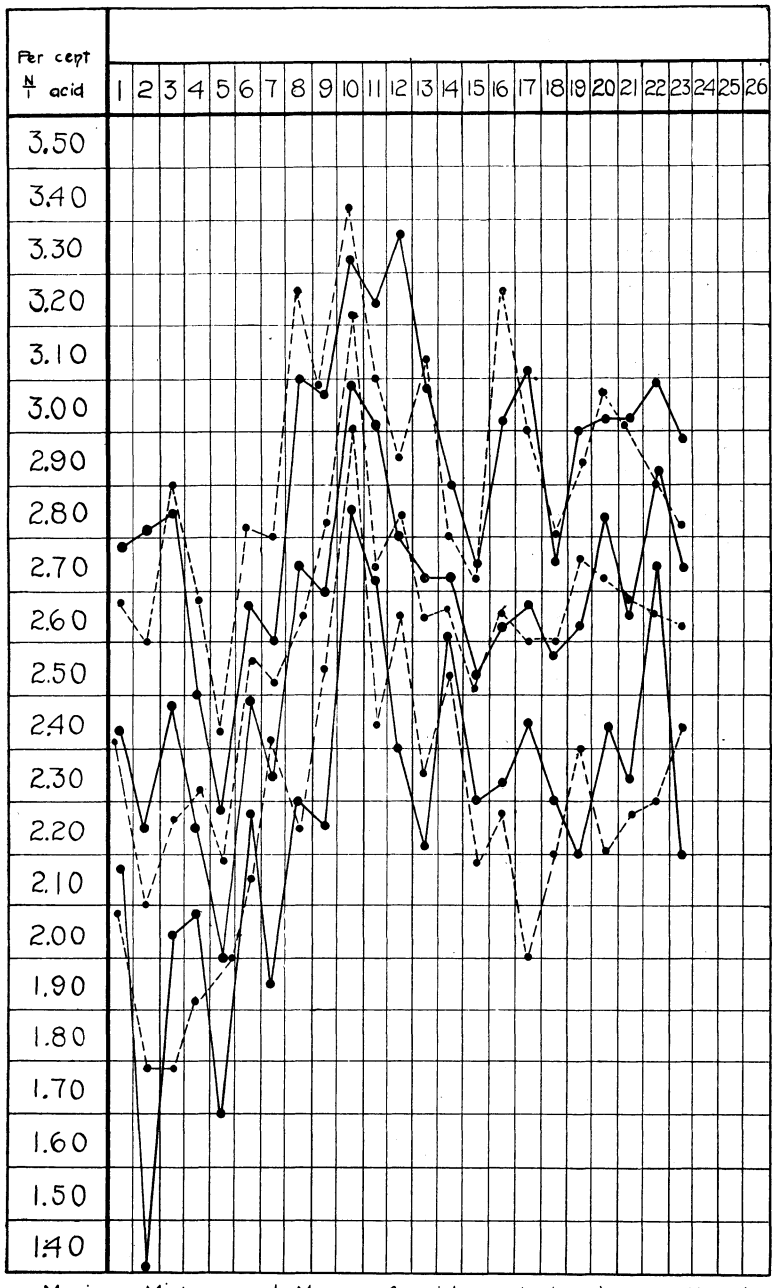


Frequency polygons showing distribution in LY, HY, LZ+HZ series.

the least. There is no evidence of any tendency toward the production of high acid and low acid races. Impressed variations of this type are evidently not heritable.

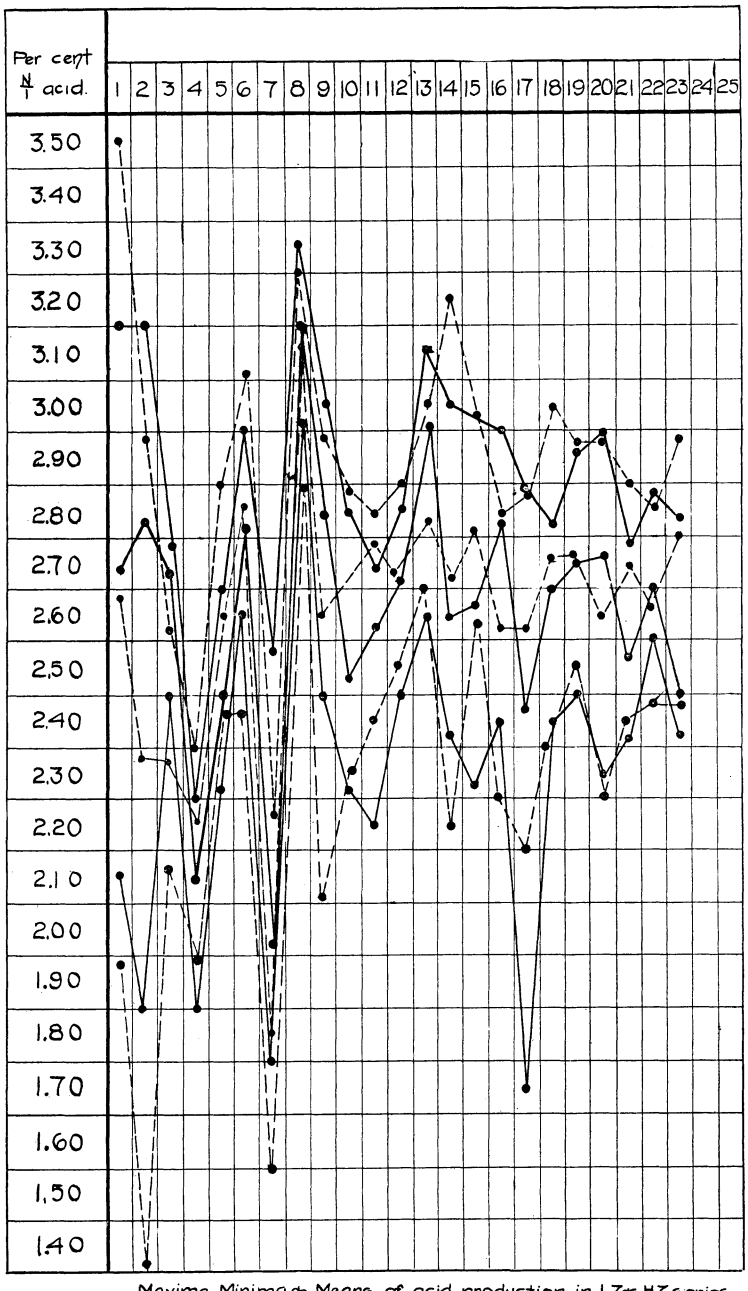
It was thought possible that 10 determinations for each series might not be a fair index to the true distribution of the acidities. To obviate this and also to secure cultures free from the immediate effect of the acid broth, litmus lactose agar plates were poured from certain members of each series. Such plates were in each instance poured from the tubes used to inoculate the next succeeding set of 10, that is, they were always from maximum or minimum acid tubes. From the plates from each tube 100 colonies were transferred to as many tubes of lactose broth, incubated, and acidity determined. Such isolations were made from the first, sixth,

CHART 4.



Maxima, Minima and Means of acid production in LY + HY series.
———=LY. ---------=HY.
Top - Maxima.
Middle - Means.
Bottom - Minima.

CHART 5.



Maxima, Minima & Means of acid production in LZ & HZ series.
Solid lines LZ – Dotted Lines- HZ.
Top - Maxima
Middle-Means
Bottom- Minima.

TABLE 3.

Percentage N/1 Acid	Frequencies																	
	Y	Z	YL_1	YH_1	ZL_1	ZH_1	YL_2	YH_2	ZL_2	ZH_2	YL_3	YH_3	ZL_3	ZH_3	YL_4	YH_4	ZL_4	ZH_4
1.0	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..
1.1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
1.2	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..
1.3	..	1	1	2	..	..	..	..	1	..	..	..	..	..	..	..	..	..
1.4	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
1.5	..	1	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..
1.6	..	..	..	3	..	..	..	..	..	..	..	..	..	..	..	..	..	..
1.7	1	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..
1.8	2	1	..	2	..	2	..	..	1	..	..	..	..	..	..	..	..	..
1.9	1	1	..	2	2	..	..	..	1	1	..	..	..	..	..	..	..	..
2.0	1	3	..	..	2	..	1	..	2	..	..	..	..	3	..	..	..	..
2.1	3	1	1	3	3	2	4	..	1	2	1	..	1	2	..	..	..	..
2.2	1	2	1	1	5	2	..	2	7	3	..	2	1	4	3	2	..	1
2.3	5	7	5	9	9	4	2	3	2	10	4	6	4	7	2	1	2	1
2.4	5	6	8	5	15	8	2	4	5	12	4	1	11	9	2	6	6	4
2.5	5	14	18	12	20	13	11	8	14	14	15	6	14	12	8	11	10	3
2.6	6	19	14	21	24	23	9	10	8	23	9	14	18	11	14	18	17	13
2.7	9	16	28	13	10	20	13	20	7	15	17	18	22	19	12	18	13	19
2.8	10	12	15	12	6	13	20	20	8	11	14	14	12	12	10	14	22	20
2.9	7	3	4	6	2	5	15	18	10	..	11	18	9	7	20	22	15	20
3.0	4	6	3	3	2	6	11	13	2	..	11	9	4	9	21	8	11	11
3.1	11	2	1	..	..	..	2	3	..	..	10	3	2	4	6	..	3	5
3.2	8	3	..	1	..	..	3	..	..	..	3	4	1	1	2	..	1	2
3.3	2	2	..	..	..	..	4	..	..	..	2	1	2	1	..	..	..	1
3.4	1	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..
3.5	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
3.6	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
3.7	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
3.8	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
3.9	3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
4.0	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
4.1	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
4.2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Total	97	101	99	97	98	98	97	101	73	91	102	96	100	101	100	100	100	100
Means	2.87 ±.036	2.60 ±.003	2.62 ±.015	2.48 ±.028	2.55 ±.016	2.61 ±.015	2.76 ±.018	2.75 ±.014	2.44 ±.028	2.53 ±.013	2.78 ±.017	2.73 ±.016	2.67 ±.017	2.64 ±.019	2.79 ±.012	2.71 ±.013	2.74 ±.013	2.79 ±.013
Standard Deviation	.5324 ±.0264	.3493 ±.0001	.2245 ±.0108	.4039 ±.0196	.2412 ±.0116	.2265 ±.0109	.2679 ±.0111	.2017 ±.0096	.3422 ±.0195	.1892 ±.0096	.2565 ±.0121	.2330 ±.0114	.2059 ±.0124	.2775 ±.0132	.1785 ±.0085	.1884 ±.0090	.1949 ±.0093	.1967 ±.0094

thirteenth, and twenty-third sets. Any tendency toward the inheritance of variations, impressed or mutative, should certainly be revealed by a comparison of results. The actual results deter-

CHART 6.

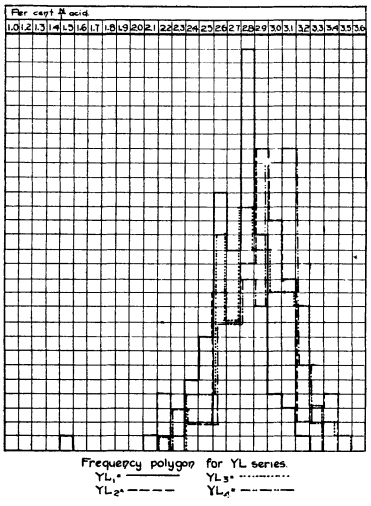


CHART 7.

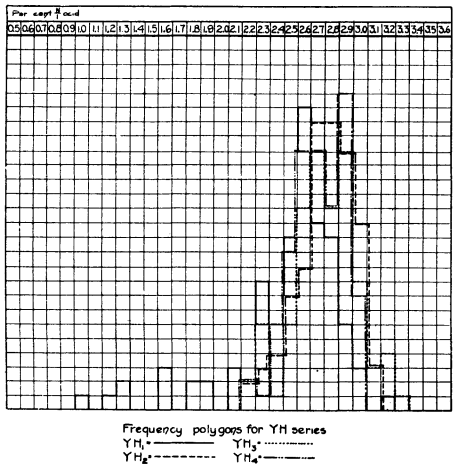


CHART 8.

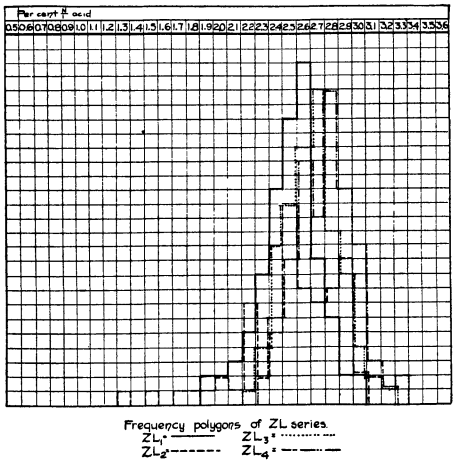
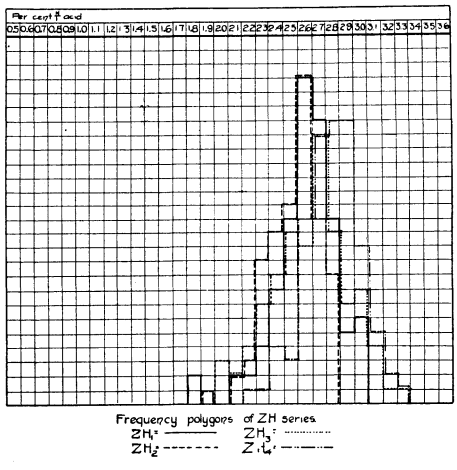


CHART 9

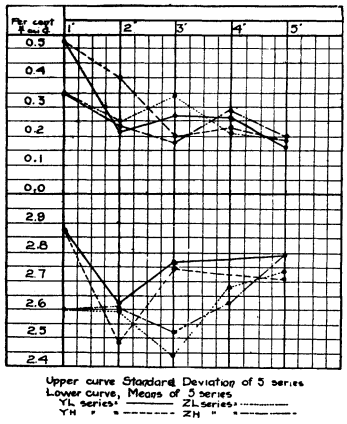


mined to the second decimal are not here recorded, but Table 3 gives the frequencies grouped in classes read to the nearest first decimal place. These series are designated YL_1, YH_1, ZL_1, and ZH_1 to YL_4, YH_4, ZL_4, and ZH_4, inclusive. The frequencies of

the original Y and Z series are also recorded. In one set, ZL_2, a part of the cultures were accidently destroyed, hence the total population of only 73 in this case. About 105 transfers were actually made in most instances, a few failed to grow, some others were lost through accidents so that in several other instances the total population recorded is less than 100. The means and standard deviations or indices of variation with probable errors are also here recorded.

The frequency polygons for the series given in Table 3 are plotted in Charts 6, 7, 8, and 9. A comparison of these curves shows that there is little or no tendency to a divergence of races. A comparison with the frequency polygons of the original Y and Z series plotted in Chart 2 indicates that there has been no material change from the original type.

CHART 10.



Upper curve Standard Deviation of 5 series
Lower curve, Means of 5 series
YL series, ——— ZL series, ———
YH " " ------- ZH " " ———

These four charts are summarized in Chart 10 by plotting the means and the standard deviations of each of the populations compared. In the diagram showing variation in means there is absolutely no evidence of a divergence of Y from Z or of LY from HY or LZ from HZ. The diagram plotting the curve of the standard deviations shows that the cultures are markedly less variable at the conclusion of the experiment than at the beginning. This may be due to a better habituation of the organisms to the medium in which they were grown, but no data are directly available on this question. The means and the standard deviations are also seen to approximate much more closely in the last transfers of the four series than in any of the preceding.

DISCUSSION.

The method here followed to test the inheritance of fluctuating and other variations is unsatisfactory in certain respects. There

is no way in which it can be determined whether heritable changes observed are due to mutation and the multiplication of the mutant and the crowding-out of the higher or lower acid forms by a gradual process of mass selection or whether changes observed are developed in all the individuals equally (mass variation). Burri believes the latter to be true with reference to indol production in the B. coli, altho his conclusions are not in agreement with other workers. Winslow and Walker obviated this difficulty by never subjecting the original cultures to the carbohydrate and acid environment, and by using the original cultures to determine mutations. The conclusions reached by them in the study of the two races of the paratyphoid bacillus agree very well with the result of the experiments recorded in this paper. Since no heritable variations could be induced in Strept. lacticus speculation as to methods and manner of variation is useless.

It should be noted that in all cases transfers were made from *acid* cultures to *neutral* broth, the organisms were therefore subjected alternately to favorable and unfavorable growth conditions. This work really amounted then to an attempt to secure two races of organisms respectively immune and susceptible to the deleterious influences of excreted metabolic products, and races that could withstand repeated transfers from a medium high in acid to another low in acid. We have no data that would determine with certainty whether or not the acid produced is the principal inhibiting factor in the growth of the organism, but it is very probable that it is such a factor. The problem of producing a high acid strain of Strept. lacticus might profitably be attacked by making repeated transfers to a medium having higher initial acid content. In this way it might be determined whether the acid-resisting powers of the lactic acid organism could be increased, and if so, whether or not acid resistance may be correlated with ability to produce acid. The differentiation of "mass change" from mutative changes would be a difficult problem, for the individual bacteria must needs then be isolated by plating out or by Barber's capillary pipette method.

Jennings compares the reproduction in protozoa to the dissolving of a crystal and to its recrystallization. "The young reappear in the form typical for the race without regard to the individual

peculiarities of the parent." While the former statement is scarcely true morphologically for bacteria as the cell wall is not resorbed during fission, nevertheless, the general statement may be said to hold, at least in the physiology of the bacteria.

SUMMARY.

An effort was made to secure high and low acid races of Strept. lacticus by constant selection and transfers of cultures from those tubes of lactose broth showing the highest and lowest acid content respectively. A comparison of these cultures carried through 23 transfers shows no evidence of a divergence of high and low acid races. These results were checked by isolation on plates from various cultures and plotting the acidity curves for populations of about one hundred. The results so obtained showed even more conclusively the fact that the variations observed are not inherited. There is no tendency to a divergence of the means. The cultures were distinctly less variable after a time, as indicated by a comparison of the standards of deviation.

It would seem that the simplest method of securing high and low acid races of Strept. lacticus would be to select from a great number of sources in an effort to secure such races already established. An effort to breed in the manner here outlined by selection would be futile. Jennings's statement, "We find that in a pure race of infusoria all the differences between individuals are environmental and without significance in inheritance," appears equally true with reference to the bacteria studied. The conclusion of Pearl and Surface, "It is found in actual experience impossible to bring about by selection improvement beyond the point already existing in the pure (isolated) strain," applies equally well to lactic acid bacteria.

CONCLUSIONS.

1. Selection as practiced under the conditions of this investigation failed entirely to fix high and low acid races of Strept. lacticus. Impressed variations do not appear to be heritable.

2. Continued growth of Strept. lacticus under favorable conditions seems to render the organism less variable.